PATENT APPLICATION
Navy Case No.: 79,212

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of: Singh et al.
Serial No.: 09/725,309
Filed: 11/29/2000
For: PASSIVATION OF NERVE AGENTS BY SURFACE MODIFIED ENZYMES STABILIZED BY NON-COVALENT IMMOBILIZATION ON ROBUST, STABLE PARTICLES
Examiner: Hutson, Richard
Art Group Unit: 1652

Honorable Commissioner of Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.131 OF ALOK SINGH, MICHAEL A. MARKOWITZ, MEHRAN PAZIRANDEH, PAUL E. SCHOEN, AND J. MATTHEW MAURO

Sir:

We, Alok Singh, Michael A. Markowitz, Mehran Pazirandeh, Paul E. Schoen, and J. Matthew Mauro, hereby declare that:

1. We are each a co-inventor of the invention claimed in the above-identified patent application.

2. Attached are copies of pages from Alok Singh's laboratory notebook titled "FY 96, 97 & 98."

3. On page 80 of this notebook, on 06/02/1997, it is documented that enzymes had been immobilized on polymerized vesicles (lines 5-6). The conception of using polymerized vesicles for decontamination or passivation of nerve agents (lines 1-2), of adding histidine sites to thioesterase (lines 11-12), and of using other substrates for enzyme immobilization (lines 17-18) is also documented

4. On page 120 of this notebook, on 03/16/1998, is documented the use of silica modified with IDA (lines 9-10).

5. Attached are copies of pages from Michael A. Markowitz's laboratory notebook titled "NB MM6."

6. On page 29 of this notebook, on 03/10/1998, The conception of immobilizing enzymes with accessible histidine groups on the surface of silica particles, binding the histidine groups to copper ion-IDA salts is documented (lines 12-17).

7. On page 31 of this notebook, on 04/07/1998, the actual preparation of copper-ion bound IDA-modified silica particles is documented. On page 33 of this notebook, on 04/09/1998, the binding of thioesterase to these particles is documented.

8. Also attached is a portion of our invention disclosure, which was complete when signed by all inventors on 04/21/1998. The disclosure states the concepts of genetically engineering thioesterase to include a poly-His tail (p.2, lines 10-11) and attaching the thioesterase to copper-IDA groups on the surface of silica particles (p. 2, lines 17-18). The examples describe in detail work that was performed to produce the genetically engineered thioesterase, Cu-IDA silica particles, and immobilized enzyme on the particles, as well as measuring the catalytic activity of various species on a contaminant.

9. All of the work cited above was performed in the United States or in a NAFTA or WTO member country.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

08/23/04
Date

Alok Singh
Alok Singh

09-23-2004
Date

Michael A. Markowitz
Michael A. Markowitz

Date

Mehran Pazirandeh

Date

Paul E. Schoen

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_____	_____
Date	Alok Singh
_____	_____
Date	Michael A. Markowitz
8-23-04	
Date	Mehran Pazirandeh
_____	_____
Date	Paul E. Schoen

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Date	Alok Singh
Date	Michael A. Markowitz
Date	Mehran Pazirandeh
30 Aug 04 Date	Paul E. Schoen Paul E. Schoen

PATENT APPLICATION
Navy Case No. 79,212

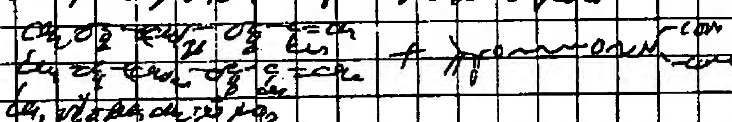
9/8/2004
Date

J. Matthew Mauro

6/2/97

Idea of using polymerized vesicles for the containment or [illegible] of nerve agents has reached a stage where it becomes necessary to explore it [illegible] on the real agents. I have previously [illegible] enzymes and polym. vesicles - now question is - if these polym. vesicles with protein binding sites can be used to extract & immobilize these proteins which hydrolyze nerve agents. A team is needed - so I initiated talks with [illegible] [illegible] who does site specific mutagenesis. I [illegible] for him [illegible] or phosphotriesterase which are modified by adding histidine sites on the enzymes. I talked to [illegible] also (indirectly) who is [illegible] expert.

— idea was [illegible] by using metal chelate polymerizable lipid for [illegible] of [illegible] [illegible]



— Planned to talk to [illegible] & Paul if other [illegible] could be used for enzyme immobilization.

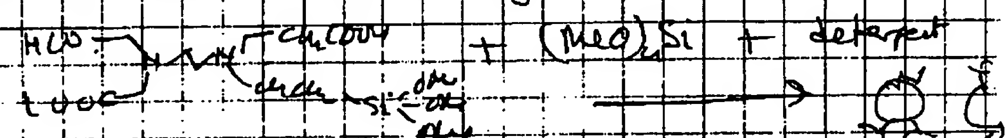
3/16/98

I held several discussions with Mike Markowitz, Mark & Paul + independently with Matt Murro about the use of ~~gene~~ cloned enzymes ~~in~~ immobilization technology for decontamination + synthesis:

Enzymes: thioesterase (made by Matt) + we are planning to make few more esterases.

Immobilization. Use IDA site + polymerizable type

Paul + Mike suggested to use silica modified with IDA, but then we agreed to make silica with IDA sites by microemulsion technique



Application

Synthesis :- Detoxification is uncontrolled but effective hydrolysis of nerve agents

But if we are using chemical agent candidates precursors + producing harmless product to be used as intermediate for other application then we are making synthetic intermediate

Liposomes good for — detoxification being tough, stable, dispersion.

Silica spheres are good — synthesis + controlled detoxification

We have decided to write a proposal (6.1) for 6.2 ONR research — Keith Ward, A. C. Palmisano, M. S. Br

Concept of Non-covalently Immobilizing Enzymes on Silica Particles

Silica particles with a wide range of diameters can be prepared using the Stöber process. The surface of such silica particles can be functionalized (-OH, NH_2, SH, vinyl, -CH=CH, etc) in at least 2 ways. One method is to modify the surface with alkoxysilanes with functionalized terminal groups. Another way to accomplish this is to co-hydrolyze the functionalized alkoxysilane with TEOS or TMOS. Using either method, immobilizing end groups can be expressed on the surfaces of silica particles. Enzymes with accessible histidine groups can then be covalently non-covalently immobilized on the surfaces of the silica particles binding the enzyme histidine groups to Copper ion - IDA delta (IDA = iminodiacetic acid) already present on the surface of the particles. Immobilization of enzymes on vesicle surfaces through non-covalent binding to surface copper ion-IDA groups has already been demonstrated ("Enzyme Immobilization on Polymerizable Phospholipid Assemblies", A. Singh, M. A. Markowitz, L. T. Taylor, J. Deschamps, in Diagnostic Biosensor Polymers, A. M. Usmani, N. Akmal (Eds.), ACS, Washington, D.C., 1994, pp 252-263.). Since enzymes immobilized onto silica particles can be packed into a variety of chromatography rods (liquid, HPLC, etc) they readily lend themselves to simultaneous continuous flow processing of multiple toxic agents.

Michael Markowitz
5/10/98

Witnessed:

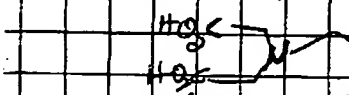
5-8-98

Michael Markowitz
NB MM6 P29

4/7/98 31

Preparation of Copper Ion-Bound IDA-Modified Silica Particles

Silica particles prepared by co-hydrolyzing TMOS with an IDA alkoxysilane derivative (see below)



were received from Paul Schoen.

The particles were then washed with ethanol in the following manner: Particles were suspended in 16 mL EtOH & divided into two equal parts. Each part was added to a test tube & then the suspensions were centrifuged (5,000 rpm, 3 min). The supernatant was decanted from each test tube. This procedure was repeated with EtOH (1 time), then with acetone (2 times).

8 mL of 2% aq $CuSO_4$ was added to each sample & the particles were resuspended with mild sonication. The suspensions were centrifuged (5,000 rpm, 3 min) & the supernatant was pipetted off. This procedure was repeated once more. These particles were then washed with distilled R.O. water. The particles were suspended in the H_2O by mild sonication, centrifuged (5,000 rpm, 3 min) & the supernatant was removed. This washing procedure was repeated 5 times.

The particles were then washed in a similar fashion with acetone. The acetone-washed particles were air-dried.

Michael Markowitz
NB MM6 P31

4/2/96

Binding of Thermolysin to Cu^{2+}-IDA Silica Particles

8 ml of 0.05 M potassium phosphate buffer (pH 7.2) was added to Cu^{2+}-IDA silica particles (MM6.31) & 8 ml of the above buffer was added to IDA-silica particles (no Cu^{2+}, MM6.32). The particles were suspended by mild sonication, centrifuged (5,000 rpm, 3 min), & the supernatant was pipetted off. This procedure was repeated one more time. 1 ml of the phosphate buffer was added to each sample & the particles were suspended with mild sonication.

40 µl of each suspension was added to 4 test tubes. Then 160 µl of the phosphate buffer was added & the resulting suspensions were rolled in an ice bucket for 5 min. Then 10 µl of the thermolysin soln (provided by Matt Nauss) was added to each test tube. The samples were incubated at 3°C for 3 hrs. Then, 50 µl of each sample was pipetted into a separate test tube. The remaining particle soln (the particles had settled out) were diluted with 3 ml of the phosphate buffer, centrifuged (5,000 rpm, 3 min, 4°C) the supernatant was pipetted off. This washing procedure was repeated 3 times. All operations were performed at 4°C.

To determine the wt of silica in 40 µl of each stock suspension (used in the digestion), 40 µl aliquots of each suspension were added to separate, pre-weighed test tubes which were then placed in an oven overnight to dry. The test tubes were then weighed & the particles were removed by resuspending them in H2O & pipetting away the suspension. The test tubes were rinsed with acetone, dried under a stream of N_2 & reweighed.

After the samples were washed, the particles were resuspended in buffer (Enzyme-particle pellets were kept wet at all times during the exp.). Final vol = 1 ml. In addition, 40 µl of Cu^{2+}-IDA silica particle stock soln was pipetted into a test tube & diluted to 1 ml with phosphate buffer.

Michael Markowitz
NB MM6 P33

PART II. DISCLOSURE OF THE INVENTION

Describe the invention fully and completely, using the outline given below.

1. GENERAL PURPOSE: (state in general terms the purpose and objects of the invention)

To provide a simple and an efficient detoxification/decontamination systems for nerve agents, which has long term stability over a wide temperature range. The object of the invention is to provide: 1) surface modified nerve agent detoxifying enzymes; 2) optimized enzymes with sustained high catalytic activity, 3) stabilized enzymes immobilized on polymerized vesicles and /or inorganic surfaces for field delivery. This invention teaches the production of efficient, easy to use and store, and environmentally friendly decontamination system.

BACKGROUND:(describe old methods, materials or apparatus used to perform the object in the invention and give their illustrations and disadvantages):

Whether due to an accidental or deliberate release, nerve agents pose a growing threat to society. The current means to counter such threats, though temporarily effective, are not adequate. Currently, activated charcoal is used in filtering these agents from air and water; bleach solution or jet fuel is used for decontaminating protective gear. The delivery of active enzyme systems to counter and detoxify chemical and biological warfare agents is a promising and active area of research. While enzymes in their native form have shown effectiveness against nerve agents, challenges exist for the development of detoxification systems, including preservation of high catalytic activity in real conditions, stability of the enzyme system after prolonged storage, suitable means of delivery, and accessibility of enzymes to threat agents. The current state of the art for detoxification with special reference to US Army's efforts has been recently reported (Detoxifying Nerve Agents, C&E News Sept.15, 1997; p.26). A class of enzymes that is known to catalyze the hydrolysis of organophosphate (OP) compounds has been investigated for potential decontamination. These OP anhydrolases (OPAA: EC 3.1.8.2) catalyze the hydrolysis of many G-type chemical warfare nerve agents. Specifically they have activity against compounds such as sarin, soman and GF (O-cyclohexyl methylphophono- fluoridate). Covalent linkage of enzymes to solid substrates and embedding enzymes in polymer matrices are the two most common means of enzyme immobilization. The covalent chemistry required for linkage to the substrate often adversely affects enzyme activity and, enzymes embedded in polymer matrices are not accessible freely to the agents present in the surrounding medium. Recently Le Jeune and coworkers have reported immobilization of phosphotriesterases onto polyurethane polymers for decontamination purposes (Le Jeune, K.E., Mesiano, A.J.,

Bower, S.B., Grimsley, J.K., Wild, J.R. and Russell, A.J., Biotechnology and Bioengineering 54:105-114, 1997). The use of polyurethane for this purpose has several drawbacks, however. In addition to not being an environmental friendly polymer, polyurethane may not afford the maximal protein stability that can be achieved in the protein?s native environment. In addition, the enzymes currently being used for these studies have not been selected for use under field conditions and suffer many drawbacks such as inhibition by substrate, low turnover, and low stability. Watkins et al. (Watkins, L.M., Mahoney, H.J., McCulloch J.K., Raushel F.M., J. Biol. Chem. 272:25596-25601,1997) have demonstrated enhanced rate of hydrolysis for phosphorus-fluorine bonds by phosphotriesterases through the use of engineered enzymes.

This disclosure addresses the deficiencies currently existing in this area. It describes a mutagenesis and selection/screening approach to obtain enzymes with the desired catalytic and stability properties. These enzymes will be further modified for non-covalent but effective immobilization on the surface of polymerized vesicles. This immobilization technique has been developed and patented at NRL (A. Singh; Liposomes containing polymerized lipids for non-covalent immobilization of enzymes and proteins; US Patent # 5663,387 Sept. 2, 1997; US Patent# 220,124 March 30, 1994). Polymerized liposomes are the prime substrate for the immobilization of active enzymes because they retain their structural integrity in adverse chemical and physical environment, provide a native environment for enzymes to sustain their activity, and provide higher surface area to facilitate easy access of medium to enzymes.

Silica particles also have high surface area and retain their structural integrity in adverse chemical and physical environments and can be used as substrates for non-covalent enzyme immobilization. Silica particles with surface IDA groups can be formed in either of two ways: 1) Silica particle precursors such as TEOS or TMOS can be co-hydrolyzed with IDA-modified alkoxysilanes using the Stober process (Stober, W., Fink, A., and Bohn, E., J. Colloid Interface Sci., **26**, 62 (1968). Alternatively, IDA alkoxysilanes can be grafted to the surface of silica particles utilizing well established literature procedures (Badley, R. D., Ford, W. T., McEnroe, F. J., and Assink, R. A., Langmuir, **6**, 792, 1990); Van Blaaderen, A., and Vrij, A., Langmuir, **8**, 2921, 1992). After forming the nickel or copper ion-IDA salt, noncovalent enzyme immobilization can proceed as previously described. Since, enzymes immobilized onto silica particles can be packed into a variety of chromatography columns (liquid, HPLC); they readily lend themselves to simultaneous continuous-flow catalytic processing of multiple toxic agents. To the best of our understanding surface modified enzymes have not been previously immobilized on silica particles.

Using either substrate, liposome or silica particle, customized immobilization protocols may be easily developed and optimized for the storage of such systems under otherwise adverse conditions. Studies are underway on the modifications of various enzymes and/or substrate components.

3. DESCRIPTION AND OPERATION (describe completely and clearly and give details of operation and use):

Our approach consists of the following four steps;

- enzyme selection,
- modification by incorporating anchor sites for linking it to target surface without losing its catalytic activity,
- stable liposome construction to accommodate and bind the selected enzymes, and
- non-covalent linking of enzyme to the colloids of surface copper or nickel IDA groups.

A thioesterase has been selected for noncovalent linkage to polymerized liposomes and silica particles. The enzyme has been genetically engineered to include poly-His tail as well as other stabilizing amino acid substitutions. Noncovalent enzyme immobilization on polymerized liposomes has been achieved by co-polymerizing amphiphiles containing metal salts of iminodiacetic acid with other polymerizable amphiphiles and then binding the enzyme to the IDA-metal salts on the outer surfaces of the vesicles. This technique relies on the strong binding affinity between copper or nickel iminodiacetate and histidine, which has been made available on the surface of the enzyme selected for immobilization through gene.

The thioesterase has also been non-covalently attached to copper-IDA groups on the surface of silica particles formed by co-hydrolyzing TMOS with an IDA-alkoxysilane derivative. The IDA-alkoxysilane accounted for 5-wt. % of the total silica content. After particle synthesis using the Stober procedure, the copper salt of the surface IDA groups was formed by adding an aliquot of 20 % aq. $CuSO_4$ solution (wt/wt) to the dry particles and then suspending the particles using mild sonication or vortex mixing. The suspension was centrifuged and the supernatant removed. This procedure was repeated and then the resulting blue silica particles were washed with water by adding the water to the particles, suspending the particles in solution, and then centrifuging the suspension and removing the supernatant. This procedure was repeated 3 times. Then, an aliquot of the thioesterase in 0.05 M phosphate buffer (pH 7.2) was added to a suspension of the particles in the same buffer. The suspension was incubated at 4 °C for 3 hrs and then the particles were centrifuged and the supernatant was

removed. The particles were then washed in using the phosphate buffer as described above. All operations involving the enzyme were performed at 4 °C. After the final washing the particles were resuspended in the buffer and stored for future use. The activity of the immobilized enzyme was confirmed using standard procedures.

Examples:

1. Cloning and Modification of Thioesterase

The gene for thioesterase-I (TE-1) of *E. coli* strain JM109 was cloned using a modification of a published procedure. Briefly, amplified DNA encoding the TE-1 protein and appropriate flanking nucleotide sequences was ligated into the DNA vector PCR 2.1 (Invitrogen). After preparation of 140 ug of the PCR2.1-TE1 vector DNA from 100 ml overnight culture, the engineered TE-1 fragment was liberated from the intermediate vector by digestion of 10 ug of this DNA with 20 units each of the restriction endonucleases NdeI and XhoI at 37°C overnight. The liberated TE-1 coding fragment was purified electrophoretically on a 2% agarose gel. The stained gene fragment was excised from the gel and subsequently obtained free of agarose using commercial products (Qiagen).

The gene for N-terminal polyhistidine-modified TE-1 was prepared by enzymatically ligating approximately 300 ng of the described gene fragment (see above) with ca. 100 ng of pProEx-1 vector DNA (Life Technologies) previously digested with NdeI and XhoI enzymes and dephosphorylated with calf intestinal alkaline phosphatase. Transformed *E. coli* DH5αF'LacI^q cells (Life Technologies) were screened for the presence of the TE-1 inserted gene by elelctrophoretic analysis of differential whole-cell protein profiles of cells taken from small scale cultures grown plus and minus 1 mM isopropylthiogalactopyranoside (IPTG) chemical inducer.

TE-1 was purified from 100 ml cell culture (LB/50 micrograms/ml carbenicillin) induced at 30°C with 1mM for ca. 2 hr (OD_{600} at induction = ca. 0.6). Cell resuspension, sonic lysis, and chromatographic purification were carried out according to published procedures. The final eluted TE-1 product (15 ml) was dialyzed for 3 days against 3 L 50 mM potassium phospate buffer (pH 7.2). The dialyzed product was concentrated in two stages to 0.65 ml using Centriprep-10 and Centricon-10 centrifugal concentrators at 4°C. The final protein concentration 0.35 mg/ml was evaluated against bovine serum albumin standard protein using a Bio-Rad (Bradford method) assay kit.

2. Assay of Enzymatic Activity Immobilized TE-1

Samples of TE-1 immobilized on IDA silica were assayed for their ability to hydrolyze p-nitrophenyl propionate (SIGMA) according to published procedures. In a typical assay, equivalent amounts of silica/enzyme slurry (or appropriate control samples) in 10 to 20 microliters were added to a 1.5 ml polypropylene conical microcentirfuge tube that contained 0.97 ml physiologically buffered saline (PBS) (pH 7.2), 3% v/v acetone, and 0.370 mM p-nitrophenyl propionate. Each tube was capped, oriented on its side, and shaken at 225 RPM at 30°C for 30 min. After 30 min, each sample was immediately centrifuged at room temperature for exactly 1 minute. Then 0.90 ml of each sample was removed and immediately assayed spectrophotometrically at 346 nm. In one such assay, the background corrected results were as follows:

Sample	Activity (OD_{346} units/min × 10^3)
Cu^{2+} + IDA silica + TE-1	5.12
Cu^{2+} + IDA silica	0.47
IDA silica	0.67

3. Formation and Catalytic Activity of Cu^{+2}-IDA Silica particles

The silica particles were formed by co-hydrolyzing TMOS with an IDA-alkoxysilane. The IDA-alkoxysilane accounted for 5-wt. % of the total silica content. After particle synthesis using the Stober procedure, the copper salt of the surface IDA groups was formed by adding an aliquot of aqueous 20 % $CuSO_4$ solution (wt/wt) to the dry particles and then suspending the particles using mild sonication and vortex mixing. The suspension was centrifuged and the supernatant removed. This procedure was repeated and then the resulting blue silica particles were washed with water by adding the water to the particles, suspending the particles in solution, and then centrifuging the suspension and removing the supernatant. This procedure was repeated 3 times. A small portion of these particles was further washed with an aq. sat. EDTA tetrasodium salt solution in a similar manner. Upon adding the EDTA solution the supernatant turned from clear to blue and the particles turned from blue to white demonstrating that copper ions had been bound to the IDA groups on the surfaces of the particles.

Then, the Cu^{+2}-IDA particles were suspended by mild sonication and vortex mixing in 1 mL of 0.05 M aq. phosphate buffer (pH 7.2). 40 μL of this suspension was added to a test tube. 160 μL of the buffer was added and the resulting suspension was cooled to 4 °C. After 3 hrs. At 4 °C, the catalytic activity of the particles was tested using a thioesterase assay. The particles exhibited catalytic activity as follows: Cu^{2+} + IDA silica particles shows an activity of 0.47 OD_{346} units/min × 10^3 and IDA silica particles showed an activity of 0.67 OD_{346} units/min × 10^3. This example demonstrates that the Cu^{+2}-IDA particles have no catalytic activity in the absence of bound thioesterase.

4. Binding and Catalytic Activity of Thioesterase on Cu^{+2}-IDA Silica Particles

The polyhistidine tagged thioesterase was noncovalently attached to copper-IDA groups on the surface of silica particles made in example 1 in the following manner: 40 uL of the suspension of the Cu^{+2}-IDA silica

particles in 1 mL of 0.05 M aq. phosphate buffer (pH 7.2) suspension was added to a test tube. 160 μL of the buffer was added and the resulting suspension was cooled to 4 °C. Then, 10 μL of the thioesterase in the phosphate buffer was added to this suspension which was then incubated at 4 °C for 3 hrs. Then, the particles were centrifuged and the supernatant was removed making sure that the silica did not go dry. The particles were washed using the phosphate buffer as described above. 8 mL of the buffer was added to the particles, which were then suspended with mild sonication, centrifuged, and the supernatant was removed. This washing procedure was repeated 6 times. All operations involving the enzyme were performed at 4°C. After the final washing the particles were resuspended in 1 ml of the buffer and stored for future use. The activity of the immobilized enzyme was confirmed using standard procedures. This sample, Cu^{2+} + IDA silica + TE-1 showed an activity of 5.12 OD_{346} units/min × 10^3. This example demonstrates sustained activity of polyhistdine modified thioesterase bound to the Cu^{+2} -IDA groups on the silica particles.

5. Binding and Catalytic Activity of Thioesterase on IDA Silica Particles.

The Cu^{+2}-IDA silica particles that had been washed with sat. Aq. EDTA (tetrasodium salt) solution were resuspended in 1 mL of 0.05 M aq. phosphate buffer (pH 7.2). 40 μL of the suspension of this suspension was added to a test tube. 160 μL of the phosphate buffer was added and the resulting suspension was cooled to 4 °C. Then, 10 μL of the thioesterase in the phosphate buffer was added to this suspension which was then incubated at 4 °C for 3 hrs. Then, the particles were centrifuged and the supernatant was removed making sure that the silica did not go dry. The particles were washed using the phosphate buffer as described above. 8 mL of the buffer was added to the particles, which were then suspended with mild sonication, centrifuged, and the supernatant was removed. This washing procedure was repeated 6 times. All operations involving the enzyme were performed at 4 °C. After the final washing the particles were resuspended in 1 mL of the buffer and stored for future use. The catalytic activity of these particles, as determined by the thioesterase assay, was significantly less than the activity of the enzyme bound to the Cu^{+2} -IDA particles. This example demonstrates that binding of the enzyme to the Cu^{+2} -IDA groups on the silica particles is required for optimal catalytic activity.

4. ADVANTAGES AND NEW FEATURES: (State the advantages of the invention over the old methods described in paragraph #2 above and the features believed to be new).

Because of additional non-military commercial potential for decontamination technology, the system disclosed here has financial benefits. Liposome and silica particle synthesis and scale-up and, enzyme engineering belong to

a class of feasible technologies. An infrastructure for commercialization exists. Relatively higher cost of lipids and engineered enzymes may be offset by accessibility of enzyme to nerve agent, higher enzyme activity, and stability in conventional storage conditions. A potential exists for making this technology commercially feasible.

5. ALTERNATIVES: Indicate any alternative methods, materials or construction of the invention.

This invention provides an effective system that utilizes the efficiency and selectivity of enzymes in catalysis and utility of surfaces to provide stability to sophisticated enzyme architecture. Other methods used in the stabilization of enzymes involve their encapsulation in polymer matrix or foams. In all these cases enzymes are subjected to chemical reactions.

7. EXECUTION OF DISCLOSURE:

Disclosed and Understood by me

Alok Singh — 4/21/98

Alok Singh — Date

Mehran Pazirandeh — 4-21-98

Mehran Pazirandeh — Date

Michael Markowitz — 4/21/98

Michael Markowitz — Date

Paul E. Schoen — 4/21/98

Paul E. Schoen — Date

J. Matthew Mauro — 4/21/98

J. Matthew Mauro — Date

Witness
JAWAD NACIRI

Witness
PAUL R. KUST